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Original Article

Serological Monitoring of Turkey Flocks After Using Avian Influenza H5 Vaccine in Iran

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Abstract

Background and Aims: Avian Influenza (AI), an acute infectious disease of waterfowl, poultry, animals, and wild birds, is transmitted zoonotically to humans. There are some reports on the HPAI incidents in Iran: H5N8 and H5N1. The Iranian Veterinary Organization decided to vaccinate turkey flocks after the outbreak in high-risk provinces in Iran. The present work aimed to evaluate the vaccine's serum response in turkey flocks in the provinces of high risks.

Materials and Methods: From Tehran (no:1), Isfahan (no: 3), Zanjan (no: 1), and Mazandaran (no:1) provinces, six broiler turkey farms were chosen and received the H5 vaccine (two times of vaccination for each farm). From each flock, 15 blood samples were taken. The HI test was conducted using 4 HA units of the homologous antigen and a U-bottomed microtiter plate.

Results: The antibody mean titers in the turkey farms previously receiving the vaccine were 1.23. However, they were 5.05 for those receiving the immunization twice (significant difference; $p<0.05$). Moreover, considering protection baseline 4, all flocks produced higher titer by injection of the vaccine twice.

Conclusion: Integrating with other control measures like good monitoring and biosecurity programs, vaccination is an appropriate and robust instrument for supporting control programs or AI eradication in endemically infected countries. The regular post-vaccination surveillance was performed by the Iranian Veterinary Organization (IVO), and the flocks were evaluated for silent infections.

Keywords: Avian Influenza, H5, Iran, broiler turkey, Vaccination

Introduction

According to FAO Statistics regarding the turkey meat production industry, Iran is in the third rank in Asia, followed by Turkey and Israel (1). Over the last decade, the industry has been developed and spread in over eighteen provinces. The consumption of turkey meat in 2007 was at 25 g per capita, and it was programmed to increase to 300-350 g by 2013 (1).

The lower infectious dose to cause the disease might explain the turkeys' higher susceptibility (almost 10-fold less viral load for turkeys infection compared to chickens) required for infection (2). Avian influenza (AI) is an acute zoonotic infectious disease in humans and birds. AIV (Avian influenza viruses) contains segmented genome located in the influenza virus A related to the Orthomyxoviridae family. Like poultry, wild birds and waterfowl, most birds are vulnerable to AI (3-6). AIVs are categorized into some subclasses in terms of

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their neuraminidase (N1–N9), haemagglutinin (H1–H16) and nucleocapsid antigens (3, 4, 7). So far, AIV subclasses producing acute disease in birds with economic importance are H7 and H5 subclasses. The birds-isolated H5 and H7 subclasses are with high pathogenicity (HPAI) and with low pathogenicity (LPAI).

H5N1 is the illustrative subclass of HPAI in Asia. It has advanced into over 32 clades characterized by their haemagglutinin (HA) genes (8-10). In China, a new H5N8 virus (Dkk1203) was identified (2010), with genes associated with the A/Goose/Guangdong/1/ 1996 lineage H5N1 lineage in birds at live bird markets. Assessing the phylogenetic tree topology, longer branches were presented by this virus compared to the formerly identified 2.3.4.1, 2.3.4.3, 2.3.4.2 subclades. The viruses were allocated to 2.3.4.4 clade as stated by the criteria of WHO/OIE/FAO) H5N1 evolution working objective group (6). Initially, isolating the HPAI A/ duck/Jiangsu/k1203/2010 H5N8 virus of the Asian H5N1 lineage (HA gene belonging to clade 2.3.4) from mallard ducks was performed, which is a live-bird market in eastern China (2010). The live poultry markets in 2013 in eastern China, were the first to isolate new reassortant H5N8 viruses. The virus was then found in poultry and wild birds in Japan and Korea.

Phylogenetic analysis revealed two distinct genetic HPAI H5N8 groups in Korea. Each group was identified as virus: A/broiler duck/ Korea/Buan2/2014- like as group A and A/ breeder duck/Korea/Gochang1/2014-like as group B. HPAI H5N8 viruses were introduced again in late 2014, into Japan and South Korea and were found in North America and Europe (11).

In Iran, there are some reports on incidents of HPAI as H5N8 and H5N1. First, the H5N1 subclass was found and approved in wild swan corpse on passive surveillance in Iran on February 13, 2006. Moreover, HPAI H5N8 was found in November 2016 on a commercial egg farm in the Tehran province. According to phylogenetic and genetic analysis of the HA gene, the Iranian H5N8 and HPAI H5N1 viruses are related to the HPAI H5 virus clades 2.3.4.4 and (2.2.1, 2.2.2, and 2.3.2.1c), respectively (3, 5, 6). Recently, vaccinating the poultry against avian influenza is a reaction to repeated outbreaks.

In the short term, vaccination campaigns are successful. However, outbreaks have recurred inevitably. The Iranian Veterinary Organization has decided Turkey flocks vaccination (H5) in high-risk provinces (Tehran, Isfahan, Zanjan, and Mazandaran) after HPAI H5N8 in Iran as a control tool. The present work aimed at evaluating the vaccine's serum response in the high-risk provinces layer flocks.

Methods

Sampling: Six broiler turkey farms were selected from Isfahan (no:3), Tehran (no:1), Mazandaran (no:1), and Zanjan (no:1) provinces that received the H5 vaccine (2 times of vaccine shots). The first vaccination age in flocks was three weeks, and the last age was six weeks. Blood sampling time was at four months of age. Fifteen blood samples were taken from each flock. Sampling details are given in Table 1.

Table 1. Turkey flocks that involved in this study (Assessment of H5 vaccination)

Farm Name	Province	Time of first vaccination (Wks)	Time of second vaccination (Wks)	Sampling Age (Wks)
1	**Isfahan**	3	6	12
2	**Mazandaran**	3	6	12
3	**Isfahan**	3	6	12
4	**Isfahan**	3	6	12
5	**Tehran**	3	6	12
6	**Zanjan**	3	6	12

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Haemagglutination inhibition (HI) test: The HI test was carried out in a U-bottomed microtitre plate, and 4 HA units of autogenous antigen in 0.025 ml phosphate buffer saline; HI tires were given titer reference number according to Kaleta and Siegmann (12).
Geometric mean titers (GMT) were calculated for each group of serum samples (Figure 1).

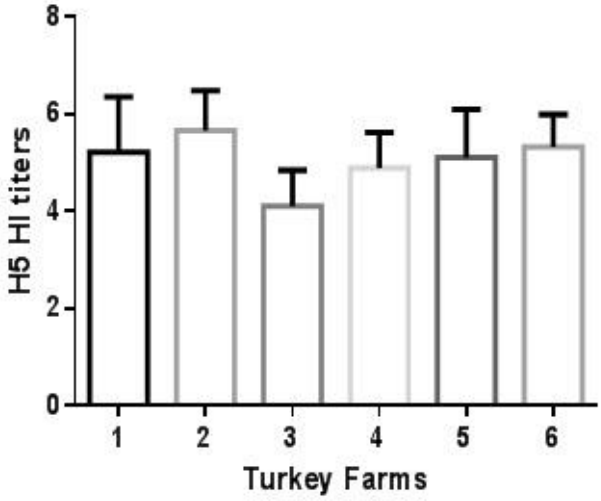

Fig. 1. The titers of HI after two vaccination.

Statically Analysis: Office Excel and Graphpad 6 software analyzed the data. The mean headline of the vaccine group was compared with the two-time t-test vaccine and the mean headline between the herds and ANOVA to compare the data. P <0.05 was considered a statistically significant level. In this study, four provinces of Iran, including Isfahan, Tehran, Mazandaran, and Zanjan, were chosen. In each province, nine farms were randomly selected. The broiler turkeys were given the h5 vaccine two times. The first vaccine is in 3 weeks and the second one in six week. At the end of the breeding period (120 days) (Table 1), 15 blood samples were given from each farm. Two ml of blood were given from each bird. The samples were transferred to the University of Tehran and were stored at 4 ℃ until examined.

Table 2. The HI titers of H5 Avian Influenza in different turkey flocks.

Farm Name (Province)	Farm1 (Isfahan)	Farm2 (Mazandaran)	Farm3 (Isfahan)	Farm4 (Isfahan)	Farm5 (Tehran)	Farm6 (Zanjan)
Titer Mean	5.22	5.66	4.11	4.88	5.11	5.33
Std. Deviation	1.13	0.81	0.73	0.73	0.99	0.66
CV(%)	21.64	14.31	17.76	14.95	19.37	12.38
Individual titer in each ≥4 (%)	88.88	100.00	77.77	100.00	88.88	100.00

Results

The mean titers in the farms before receiving the first vaccine were 1.23, while those that received the vaccine twice were 5.05 (significant difference; p<0.05) (Chart 1). Also, if we consider protection baseline 4, all of the flocks (100%) could make it above it. The lowest average titer was 4.11, and the highest average titer was 5.66. Injection of the vaccine twice also improved CV. The percentage of individual titers in each group above four was also calculated (Table 2).

Discussion

AIV is an emergent threat to public health. It continuously causes outbreaks amongst humans and poultry. Turkey is sold in Iran as meat at the age of four months. HPAIVs-infected turkeys can be found dead with no prodromal signs (13-16).
The common clinical signs of turkey flocks are increased mortality, reduced water and feed consumption, respiratory and diarrhea signs, and depression. Turkeys' most specific (79%) and sensitive (100%) signs included reduced growth performance, depression, decreased vocalization, huddling, yawning, swollen sinuses, recumbency with an extended neck, and mucosal secretions from the beak. Similarly, the most dominant gross lesions can be inconstant (14, 15, 18) and maybe different

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between chickens and turkeys infected with the same virus (17). Turkeys should be examined for HPAIV regarding higher mortality or other indications in line with this disease, even when HPAI is not specifically suggested by the necropsy results (15). The expression and onset of clinical symptoms can be different with the virus isolate and dose (19, 20). In 1941, the influenza viruses' capability in agglutinating erythrocytes was explained (21).

When influenza occurs, a lattice will be formed by an erythrocytes suspension, and sedimentation will be failed, which is haemagglutination. There is a correlation between the receptor specificity of influenza A viruses and their capability in agglutinating red cells from various animal species.

Previously, there has been less information regarding turkey's influenza vaccination under field circumstances. In laboratory studies, ducks and chickens have been protected against lethal challenges by vaccination against H5 influenza viruses (22). Factors affecting vaccination consequences are the quality and type of vaccine, vaccination dose, schedule, and administration method. Notably, no single suggested regime exists for HPAI vaccination of commercial poultry in the endemic situation. Immunity induced by vaccine is determined by existing haemagglutination inhibiting (HI) antibodies in vaccinated birds. Moreover, the efficacy of the vaccine is generally reflected by HI titers, which is correlated with protection from virulent H5N1 challenges (23).

Regarding the manual minimum HI serological titers of OIE in the field, birds should be 1/32 protected from mortality or higher than 1/128 to present a reduced challenge in virus shedding and replication.

This work revealed that it is essential to vaccinate flocks vaccinated twice. It is essential to take into account different causes of week response. The findings of this work could present a baseline of 4 on average. Baseline contributes to the veterinarians and veterinary organizations assessing the vaccine and detecting the silent infection. The number of birds protected from virulent challenges is the key feature of an effective H5N1 vaccination program, which is the "flock immunity level." The present estimation of this is that 60% of the birds and more possess HI titers of higher than 4log2 or spread of the H5N1 challenge virus is decreased or prohibited (24). In our study, all of the vaccinated flocks had HI titers of ≥ 4log2 at 16 Wks and thus were protected. Based on the results, we have silent infections. However, protective immunity was then declined, at a variable rate related to a degree, and the number of presented vaccinations. It was speculated that to ensure flock immunity, it is essential to vaccinate 90% of a flock. A high-quality vaccination is also required to elicit a permanent antibody response (25). For example, in Mexico, vaccination programs have been underway against H5N2 epizootics since 1995 (26). However, in the end, antigenic drift was caused by widespread vaccination from the vaccine strain, contributive to vaccination failure.

H5N1 vaccination programs were instituted in Southeast Asia, Hong Kong, Indonesia, Vietnam, and China. In this region, the concern is insufficient vaccine coverage. Only 20–50% of all flocks were vaccinated in China, and only 40–60% in Vietnam (22, 27). Though no outbreaks were reported in vaccinated flocks, any H5N1 virus found into these flocks may be disseminated further by vaccinated poultry-like virus shed by asymptomatically infected ducks, which is only protected against severe illness (28).

Tarigan et al. (2018) showed that the individual birds' HI titers in each flock are meaningfully different from birds in other flocks. They found that the field vaccination effectiveness was highly farm-related and variable (23). Using appropriately, vaccination protected poultry against death and clinical signs. Moreover, virus shedding was markedly reduced in vaccinated birds, decreasing virus transmission (29, 30), however full health care is initial approach to avoid novel viruses or isolates (31, 32, 33).

Based on our results, at least two vaccinations are needed for providing satisfactory titration and serum protection. Nevertheless, it is essential to vaccinate repeat-edly to obtain higher titers and avoid virus replication. Moreover, the minimum headline required for

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protection is not provided by a single vaccination. The following are suggested to improve vaccination strategy outputs:1. Sero-Screening of all vaccinated flocks to find the baseline and silent infection, 2. Antigen tracking test (Real-time PCR) and periodic sampling for flock monitoring, 3. Challenge studies to assess the vaccine's efficacy on Iranian H5 circulating strains, 4. Increasing vaccination coverage, 5-Creating a program for removing the vaccine policy, 6. Performing the HI test with Iranian H5 Antigen.

Conclusion

Integrated with other control measures like good monitoring and biosecurity programs, vaccination is an appropriate and powerful instrument for supporting AI eradication or controlling programs in countries endemically infected.

Therefore, the regular post-vaccination surveillance was performed by the Iranian Veterinary Organization (IVO), and the flocks were evaluated for silent infections.

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Conflict of interest

No conflict of interest is declared.

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References

1. Salahi A. A review of the turkey meat production industry in Iran. Zootec Int. 2014;36:24-29.

2. Pillai S, Pantin-Jackwood M, Yassine HM, Saif YM, Lee CW. The high susceptibility of turkeys to influenza viruses of different origins implies their importance as potential intermediate hosts. Avian Dis. 2010;54(s1): 522-526.

4. Ghafouri SA, Ghalyanchi Langeroudi A, Maghsoudloo H, Tehrani F, Khaltabadifarahani R, Abdollahi H, et al. Phylogenetic study-based hemagglutinin (HA) gene of highly pathogenic avian influenza virus (H5N1) detected from backyard chickens in Iran, 2015. Virus genes. 2017;53(1):117-120.

4. OIE, W., Update on highly pathogenic avian influenza in animals (type H5 and H7). Office International des Epizooties, Paris, France, 2017.

5. Ghafouri SA, Fallah Mehrabadi MH, Talakesh SF, Hosseini H, Ziafati Z, Malekan M,et al. Full genome characterization of Iranian H5N8 highly pathogenic avian influenza virus from Hooded Crow (Corvus cornix), 2017: The first report. Comp Immunol Microbiol Infect Dis. 2019;64:73-80.

6. Ghafouri SA, GhalyanchiLangeroudi A, Maghsoudloo H, Kh Farahani R, Abdollahi H, Tehrani F,et al. Clade 2.3. 4.4 avian influenza A (H5N8) outbreak in commercial poultry, Iran, 2016: the first report and update data. Trop Anim Health Prod. 2017;49(5): 1089-1093.

7. Swayne DE, Suarez DL. Highly pathogenic avian influenza. Rev Sci Tech. 2000;19(2):463-482.

8. Kim S-H, Hur M, Suh J-H, Woo C, Wang S-J, Park E-R, et al. Molecular characterization of highly pathogenic avian influenza H5N8 viruses isolated from Baikal teals found dead during a 2014 outbreak in Korea. J Vet Sci. 2016. 17(3):299-306.

9. Chowdhury S, Enayet Hossain M, Kumar Ghosh P, Ghosh S, Muhammad Belal Hossain M, Beard C, et al. The pattern of highly pathogenic avian influenza H5N1 outbreaks in south Asia. Trop Med Infect Dis. 2019:4 (4):138.

10. Lee E-K, Kang H-M, B-M, Lee Y-N, Gyeong-Beum Heo G-B, Hee-Soo Lee, et al. Surveillance of avian influenza viruses in South Korea between 2012 and 2014. Virol J. 2017;14(1):1-10.

11.Kanehira K, Uchida Y, Takemae N, Hikono H, Tsunekuni R, Saito T ,et al., Characterization of an H5N8 influenza A virus isolated from chickens during an outbreak of severe avian influenza in Japan in April 2014. Arch Virol. 2015;160(7):1629-1643.

12. .Kaleta, EF, Siegmann O. Kinetics of NDV specific antibodies in chickens—V. Analysis of frequency distributions of antibody titers against Newcastle disease virus by investigation of random samples in chicken flocks. Comp Immunol Microbiol Infect Dis. 1978;1(1-2):83-92.

13. Alexander D, Allan WH, Parsons DG, Parsons G. The pathogenicity of four avian influenza viruses for fowls, turkeys and ducks. Res Vet Sci. 1978;24(2):242-247.

14. Narayan O, Lang G, Rouse B. A new influenza A virus infection in turkeys. V. Pathology of the experimental disease by strain turkey-Ontario 7732-66. Arch Gesamte Virusforsch. 1969;26(1):166-182.

15. Elbers ARW, Fabri THF, Vries TS de, Wit JJ de, Pijpers A, Koch G. The highly pathogenic avian influenza A (H7N7) virus epidemic in The Netherlands in 2003—lessons learned from the first five outbreaks. Avian Dis. 2004; 48(3):691-705.

16. Alexander DJ, Parsons G, Manvell RJ. Experimental assessment of the pathogenicity of eight avian influenza

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A viruses of H5 subtype for chickens, turkeys, ducks and quail. Avian Pathol. 1986;15(4):647-662.
17. Perkins LE, Swayne DE. Pathobiology of A/chicken/Hong Kong/220/97 (H5N1) avian influenza virus in seven gallinaceous species. Vet Pathol. 2001;38 (2):149-164.
18. Elbers ARW, Kamps B, Koch G. Performance of gross lesions at postmortem for the detection of outbreaks during the avian influenza A virus (H7N7) epidemic in The Netherlands in 2003. Avian Pathol. 2004;33(4):418-422.
19. Narayan O, Lang G, Rouse B. A new influenza A virus infection in turkeys. Arch Gesamte Virusforsch, 1969;26(1-2):149-165.
20. Aldous EW, J M Seekings JM, McNally A, Nili H, Fuller CM, Irvine , D J Alexander ,et al., Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus type 1 viruses in chickens, turkeys and ducks. Avian Pathol. 2010;39(4):265-273.
21. Hirst GK. The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. Science. 1941;94(2427):22-23.
22. Boltz DA, Douangngeun B, Sinthasak S, Phommachanh P, Midouangchanh P, Walker D, et al. Field assessment of an H5N1 inactivated vaccine in chickens and ducks in Lao PDR. Arch Virol. 2009;154 (6):939-944.
23. Tarigan S, Haryadi Wibowo M, Indriani R, Sumarningsih S, Artanto S, et al. Field effectiveness of highly pathogenic avian influenza H5N1 vaccination in commercial layers in Indonesia. PloS One. 2018;13(1): e0190947.
24. Bouma A, Claassen I, Natih K, Klinkenberg D, Donnelly CA, Koch G, et al. Estimation of transmission parameters of H5N1 avian influenza virus in chickens. PLoS Pathog. 2009;5(1):e1000281.
25. Savill NJ, Rose SGS, Keeling MJ, Woolhouse MEJ, et al. Silent spread of H5N1 in vaccinated poultry. Nature. 2006;442(7104):757-757.
26. Villarreal C. Control and eradication strategies of avian influenza in Mexico. Dev Biol (Basel). 2006;124: 125-126.
27. Ellis TM, Leung CYHC, Chow MKW, Bissett LA, Wong W, GuanY, et al. Vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission. Avian Pathol. 2004;33(4): 405-412.
28. Hulse-Post D, Sturm-Ramirez KM, Humberd J, Seiler P, Govorkova EA, Krauss S, et al. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc Natl Acad Sci USA. 2005;102(30):10682-10687.
29. Swayne D. Vaccines for List A poultry diseases: emphasis on avian influenza. Dev Biol. 2003;114:201-212.
30. Hamad M, Amen O, Mahmoud M, Hassanin O, Saif-Edin M, et al. Effectiveness of different avian influenza (H5) vaccination regimens in layer chickens on the humoral immune response and interferon-alpha signalling immune marker. Vet Res Commun. 2018;42 (2):145-152.
31. Rezapanah MR. Establishing national coherent systems for organic agriculture and food and feed risk analysis toward sufficient food safety system. Proceeding of national conference of organic animal, poultry and aquatics products, Rasht, Iran, Sept 2-3, 2015. 15 pages. DOI: 10.13140/RG.2.1.1878.0887.
32. Meki I K, Henry K, Rezapanah MR, van der Vlugt RAA, Abd-Alla AMM, van Oers MM, Vlak JM. Characterization of Novel RNA viruses isolated from tsetse fly Glossina morsitans morsitans. International Congress on Invertebrate Pathology and Microbial Control & 52nd Annual Meeting of the Society for Invertebrate Pathology & 17th Meeting of the IOBC/WPRS Working Group “Microbial and Nematode Control of Invertebrate Pests. July 28 - August 1, 2019, Valencia, Spain.
33. Ghalyanchi Langeroudi A, Rezapanah MR, Yusefzade kalkhoran A. A quick overview of herbal medicines (phytobiotics) in the commercial poultry. Proceeding of national conference of organic animal, poultry and aquatics products, Rasht, Iran, Sept 2-3, 2015. 10 pages. DOI: 10.13140/RG.2.1.3319.8805.

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